

POSTER PRESENTATIONS

PD 05

Stool processing-methods for *Salmonella enterica* isolation and PCR detectionOLIVEIRA, C.J.B.¹, CARVALHO, L.F.O.S.², FRESCHI, C.R.², FERNANDES, S.A.³, TAVECHIO, A.T.³

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Summary: The aim of this study was to compare the efficiency of three protocols for bacteriological isolation of *Salmonella enterica* and detection by PCR in swine feces samples. Pool of feces (n=62) were processed by three different methods. Method 1: samples (10g) were pre-enriched in BPW (1:10) and enriched in Rappaport-Vassiliadis broth (1:100). Method 2: samples (1g) were first enriched in GN-Hajna broth (1:10) and secondly enriched in Muller-Kaufmann tetrathionate broth (1:10). Method 3: Single step enrichment of feces (1g) in selenite-cystine broth (1:10). PCR was performed using DNA extracted from the last enrichment broth of each bacteriological method. *Salmonella enterica* was cultured from 13 out of 62 samples (20.9%) and seven different serotypes were isolated. The methods 1, 2 and 3 resulted in 9 (14.5%), 6 (9.6%) and 2 (3.2%) positive samples, respectively. PCR was significantly superior than conventional bacteriology for *Salmonella* detection only when Rappaport-Vassiliadis was used for DNA-template preparation.

Keywords: Detection, enrichment, pigs, Rappaport-Vassiliadis, swine

Introduction: Conventional bacteriology still is the basis of epidemiological studies of *Salmonella*. However, *Salmonella* serovars are not detectable in certain clinical samples that contain small numbers of organisms. PCR is a powerful molecular biology tool for the detection of target DNA, but its application to fecal samples has been very limited because of the presence of unknown PCR inhibitors (Lou et al., 1997). The use of PCR coupled to selective enriched broths has been considered a feasible alternative to improve the sensitivity of the PCR from clinical samples because they dilute inhibitors and allow the increase of *Salmonella* organisms in the sample. The aim of this study was to compare three bacteriological protocols for *Salmonella* isolation. Additionally, the effectiveness of PCR coupled to the last enrichment broth of each protocol was also evaluated.

Material and Methods: Pool of feces (n=62) were collected from holding pens in a slaughter-house. Samples were collected using sterile plastic bags and taken to the lab under refrigeration to be processed in the same day. After homogenization, each sample was submitted to three isolation methods. Method 1 (M1): feces samples (10g) were pre-enriched in BPW 2% (1:10), incubated overnight at 37AC and transferred (1:100) to Rappaport-Vassiliadis broth (RV), which was incubated at 42AC for 24 hours. Method 2 (M2): feces samples (1g) were first enriched in GN-Hajna broth (1:10), incubated for 24 hours at 37AC and transferred (1:10) to Muller-Kaufmann tetrathionate broth (TT), which was incubated at 37AC for 48 hours. Method 3 (M3): single step enrichment of feces (1g) into 10 mL selenite-cystine broth (SC) for 24 hours at 37AC. After enrichment, a loopful of each tube was streaked onto XLT agar plates. After biochemical analysis, colonies were confirmed as *Salmonella* by slide agglutination test. Aliquots (1mL) of each enriched broth were taken and submitted to DNA extraction by a boiling-centrifugation technique. (Soumet et al., 1994). PCR was performed using specific primers for the *Salmonella* genus (S18-S19). Results were analyzed by McNemar's test for matched samples.

Results: *Salmonella enterica* was cultured from 13 out of 62 samples (20.9%) and seven serotypes were isolated: S. London, S. Lexington, S. Schwarzengrund, S. Mbandaka, S. Hadar, S. 1,3,19:-: and S.

Senftenberg. M1 resulted in 9 (14.5%) positive samples, which was superior ($P<0.05$) than M2 (6, 9.6%) and M3 (2, 3.2%), respectively.

PCR was superior ($P<0.01$) than conventional bacteriology only when RV was used as the DNA source. PCR using RV detected 27 out of 62 samples (43.5%), which was superior ($P<0.01$) than using SC or TT (3.2% and 8.0%, respectively).

Discussion: Considering the effect of sample weight on the detection of *Salmonella* bacteria (Davies et al., 2000), we cannot directly compare the three enrichment media used herein. However, we suggest the use of M1 whenever possible to obtain large amounts of fecal material (10g). Furthermore, the use of direct enrichment of swine feces in SC is discouraged.

The coupling of RV to PCR was significantly superior ($P<0.01$) than either TT or SC. Indeed, RV has been successfully used for PCR purposes in poultry-related samples (Oliveira et al., 2002). The best results of M1 (which used RV as enrichment broth) for isolation of *Salmonella* could indicate that this broth yielded higher numbers of *Salmonella* organisms, which could also explain the superior PCR results for RV. However, selective enrichment media may have different inhibitory properties to PCR. In fact, tetrathionate broth has been suggested to inhibit PCR (Stone et al., 1994).

Our results indicate that RV broth may be successfully coupled to PCR after a simple boiling-centrifugation technique for DNA extraction. This is of great importance considering the cost-benefit ratio of this procedure.

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COMPARISON OF ENRICHMENT SCHEMES FOR THE ISOLATION OF *YERSINIA ENTEROCOLITICA*

PD 06

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Summary: The food-borne pathogen *Yersinia enterocolitica* (YE) has been repeatedly linked to swine and is a world wide food safety risk. Microbiological culture methods for YE lack some functionality as the current gold standard requires a 21 day cold enrichment in phosphate buffered saline (PBS). In this study a shortened enrichment scheme using a higher incubation temperature and a more selective media (LB-BSI) was compared to PBS for the isolation of YE from swine feces. Both enrichments